The emerging concept of transportome: state of the art

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Abstract

The array of ion channels and transporters expressed in cell membranes—collectively referred to as the transportome—is a complex and multifunctional molecular machinery; in particular, at the plasma membrane level it finely tunes the exchange of biomolecules and ions acting as a functionally adaptive interface that accounts for dynamic plasticity in the response to environmental fluctuations and stressors. The transportome is responsible for the definition of membrane potential and its variations, participates in the transduction of extracellular signals, and acts as a filter for most of the substances entering and leaving the cell, thus enabling the homeostasis of many cellular parameters. For all these reasons, physiologists have long been interested in the expression and functionality of ion channels and transporters, both in physiological and pathological settings, and across the different domains of life. Today, thanks to the high-throughput technologies of the post-genomic era, the *omics* approach to the study of

the transportome is becoming increasingly popular in different areas of biomedical research, allowing for a more comprehensive, integrated, and functional perspective of this complex cellular apparatus. This paper represents a first effort for a systematic review of the scientific literature on this topic. Here we report a brief overview of all those studies—both primary and meta-analyses—that looked at the transportome *as a whole*, regardless of the biological problem or the models they used. A later section is devoted to the methodological aspect by reviewing the most important public databases annotating ion channels and transporters, along with the tools they provide to retrieve such information. Before conclusions, limitations and future perspectives are also discussed.

Introducing the transportome

The word *transportome*, a relatively new term in cell biology, is now increasingly used to refer to the entire family of membrane proteins responsible for the translocation of any kind of solutes across the lipid bilayer. More precisely, it can be defined as the set of the ion channels that mediate the influx/efflux of ions (and occasionally larger molecules) across cell membranes *plus* all the transmembrane proteins involved in the uptake/export of solutes, including electrolytes, trace minerals, nutrients, toxic substances, signaling molecules, energetic substrates, metabolites, waste products, and drugs. Conventionally, proteins implicated in paracellular channel complexes (e.g., claudins) and vesicle-mediated transport are instead kept apart. As for humans, the whole transportome accounts for about 5% of the protein-coding genome, that is to say ≈1,000 genes overall comprising ≈450 solute carriers (SLCs), ≈50 ATP-binding cassette (ABC) transporters, ≈320 ion channels (among which ionotropic receptors), ≈90 ATPase

pumps, and 14 aquaporins (AQPs), according to the HGNC gene family classification system (see *Where to find all the ICTs you need?* section below). Thus, the transportome is the functional layer that, placed at the interface between two compartments, acts as a gatekeeper for the exchange of substances, genesis of the electrochemical gradients, and the fulfillment of all the homeostatic processes that make life possible. It also plays a key role in signal transduction, as it mediates ionotropic neurotransmission, excitation–contraction coupling, and sensory transduction.

Representing the main molecular machinery responsible for transcellular fluxes and cellular excitability, ion channels and transporters (ICTs) have long been crucial in epithelial physiology, neurosciences, and cardiovascular research. Recently, even cancer research has placed great emphasis on them, being the essential prerequisite for cancer cells to survive and proliferate in non-physiological conditions like those of the tumor microenvironment (TME), frequently characterized by altered pH, dysfunctional vascularization, hypoxia, and augmented matrix stiffness (1–3). Dysregulated ion channels and/or transporters have been linked to cell cycle dynamics (4), migration (5, 6), apoptosis (7), and vascularization (8), all of which ultimately contribute to cancer progression. The modification of the resting membrane potential is another characteristic of cancer cells (9–11), so that the altered expression of the transportome was proposed as a new *hallmark of cancer* (12, 13) in addition to those originally described by Hanahan and Weinberg (2, 14, 15).

Along with and beyond their primary function (16, 17), ICTs have been linked to a multitude of diseases (18–20). Given the wide variety of well-characterized chemical compounds acting as blockers, activators, or modulators of their action, they have gained attention as both molecular biomarker (21) and promising pharmaceutical

targets (22). Indeed, ICTs are the second largest class of pharmacological targets for drugs, just after G protein-coupled receptors (23), and their relevance is set to rise in future (24). In recent years, drug repurposing has emerged as a potent and affordable method to target the transportome by the off-label application of molecules already available on the market and currently used in both clinical and preclinical research (25, 26).

The traditional—and still prevalent—experimental approach to ICTs has been based on electrophysiology, immunostaining, fluorescence labeling, and other molecular biology tools such as RT-qPCR, western blotting, etc. Although these technologies are well-established, they typically only permit the study of one (or a small number of) ICT type(s) at a time, with the choice usually being based on the expertise of each research team. Such narrow scopes, however, are likely to fail in detecting large-scale transportome alterations, involving more than just individual ICTs. Recently, the use of high-throughput (HT) technologies, such as genomics, transcriptomics, proteomics, and even multi-omics, has paved the way to a more data-driven and unbiased investigation of the transportome, allowing scientists to grasp the complexity of this multifaceted cellular apparatus.

This review is intended to provide the current state of the art about transportome, not simply viewed as a sub-set of the overall variety of proteins, but as a physiologically consistent tool playing the critical function as a mediator of the cellular adaptation to the external conditions, which requires high flexibility and dynamic features. All the research articles with an *omics* approach to ICTs that we found are discussed, focusing in particular on the reasons underlying the interest in the transportome, the methods adopted, the main findings, the contingent limitations, and the future perspectives.

Beyond the diversity of the several research fields interested in transportomics and the variability of the associated biological models, in the next sections we will try to show how the scientific focus actually has recurring themes, the main of which are schematically outlined in Figure 1.

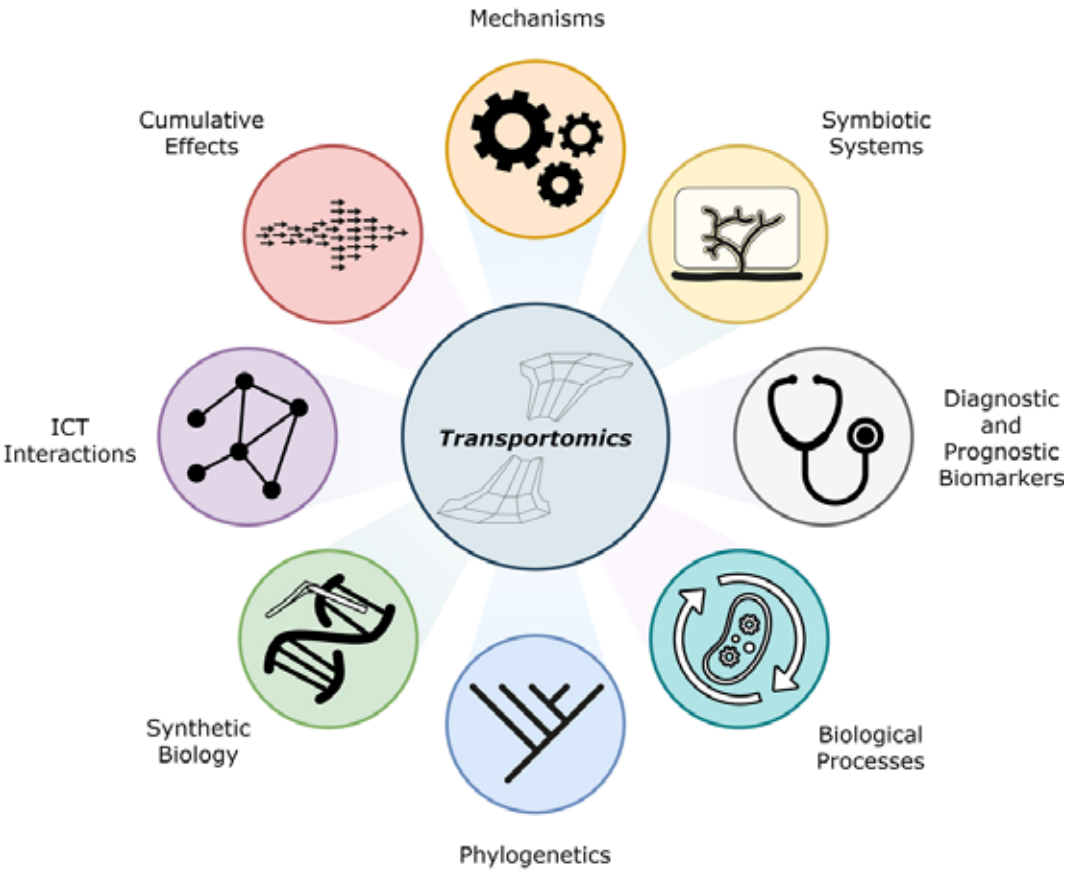

Figure 1. Recurrent themes and aims in transportome investigation. *From top, counterclockwise: gene-drug correlation analysis to unveil mechanisms of action; identification of cumulative effects of many moderately dysregulated ICT genes; mapping of the potential interactions among different ICTs or ICT families; genome editing for the industrial production of molecules of interest by recombinant microorganisms; sequencing of the expressed ICTs for subsequent phylogenetic analyses; differential expression analysis to explain relevant biological processes or establish new drug targets; ICT expression profiling in specific pathological conditions to discover new diagnostic biomarkers or to correlate with patient survival and find new prognostic biomarkers; study of interface dynamics in symbiotic systems.*

The transportome in literature

As of April 2023, a PubMed search for papers including the word *transportome* in their title or abstract returned just 82 results, including 29 reviews or editorials and 53 original research articles. Among the latter, only 34 papers were selected for further discussion since they were the only ones that actually analyzed the *whole* transportome or, at least, an entire molecular class of transporters (all SLCs, all ion channels, etc.) in an unbiased and data-driven way. In the remaining cases, the term *transportome* was used either incidentally or to indicate a relatively small subset of functionally related transporters chosen *a priori* (e.g., all the transporters for metals (27–30), zinc (31), sugar (32–34), drugs (35, 36)), indicative of a more hypothesis-driven approach. For the purpose of this review, such works were excluded as well as those few publications in which the word *transportome* was employed in a completely different sense, referring to the collection of proteins that mediate the transport of specific molecules *within the cell* as opposed to *across the cell membrane* (e.g., the mRNA transportome (37)), or, most curiously, the pool of *transported proteins itself* (38, 39).

In any case, since the term *transportome* is still not universally adopted among cell physiologists, the results returned by such an oversimple query are unlikely to exhaustively represent the state of the art about the current omics approaches to ICTs. For this reason, we extended our search using more heuristic criteria, cross references, and PubMed suggestions based on topic similarity, albeit neglecting those global omics studies that were not deemed programmatic and systematic investigations of the transportome, even when mentioning some ICTs among the many gene families discussed. Although we greatly broadened the set of works considered in this second phase, we still cannot be sure we didn't overlook any important or even noteworthy

studies, because not all ICT omics researchers refer to it as the *transportome*, just like not all discussions of the *transportome* actually refer to ICTs—though such terminology is gradually taking hold, and the meaning of *transportome* has already largely converged to the acceptation employed in this review.

Overall, here we collected a bibliography of 56 transportome-oriented research articles and 83 reviews. Even if most of these studies were published after 2010, we did not restrict our search to any period. The earliest relevant research paper we could find was published in 2003 (40), although, as early as 2001, it was possible to find some reviews that compiled all the ICT-related evidence available at that time on specific topics (41–43). A timeline of the most significant events in transportome research over the past 20 years, together with the detailed time distribution of all the transportome-related publications covered by the next section, is shown in Figure 2, highlighting the growing interest of the biomedical community in this field.

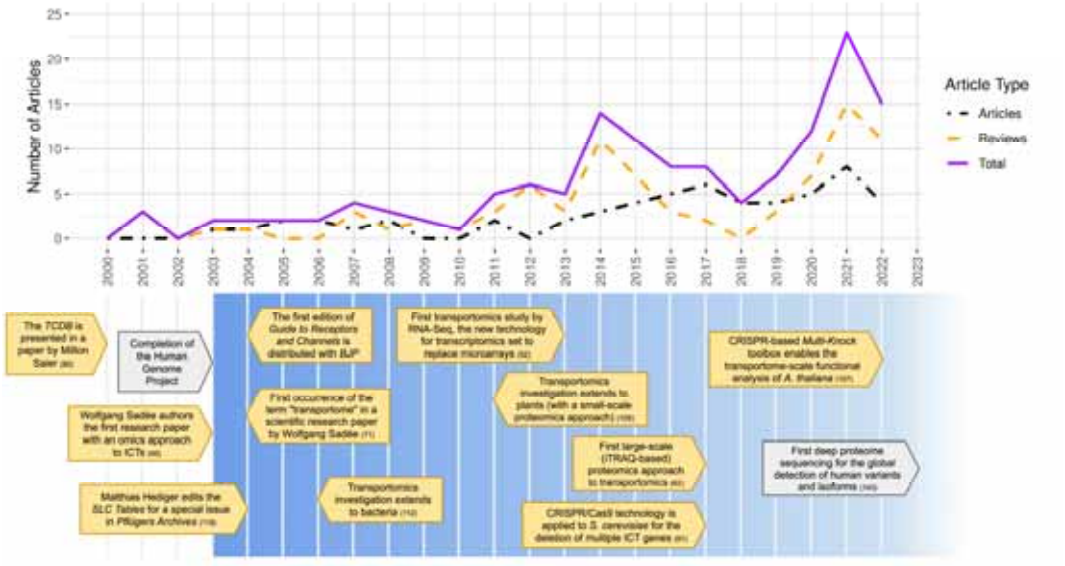

Figure 2. Transportomics timeline. *Upper: Time distribution of all the transportome-oriented publications considered in the review. The increasing trend of the publication rate per year of both primary studies or meta-analyses (dashed black line), and reviews (dashed yellow line) reflects the gradual emergence of the omics approach to the study of ion channels and transporters. Lower: timeline of the most significant events that have contributed to the definition of transportomics over the past 20 years. Bibliographic references are*

given in round brackets. TCDB: Transporter Classification Database; ICTs: Ion Channels and Transporters; SLC: solute Carrier; BJP: British Journal of Pharmacology; iTRAQ: isobaric Tags for Relative and Absolute Quantitation.

It is worth noting that most of the publications we found searching for transportome-related keywords were reviews outlining the transportome configuration in a given biological model by aggregating evidence from many low-throughput primary studies, each addressing one or few ICTs in similar pathophysiological conditions (e.g., (44)). The general recommendation is not to confuse such qualitative and manually curated lists of dysregulated ICT genes with the quantitative meta-analyses of multiple transportomics primary studies (e.g., (45)), because the "gene signatures" reported by the former are frequently plagued with severe limitations, heavily depending on the particular pool of references taken into consideration. Even when systematic, these *single-ICT pooling reviews* are likely to be intrinsically incomplete since they obviously omit all those differentially expressed genes (DEGs) that have never been reported, perhaps only because they fall outside the primary lines of inquiry. Moreover, despite addressing the same problem in the same biological model, the primary studies under consideration are rarely perfectly comparable due to a variety of boundary experimental conditions (such as culture time, treatment, sample processing, tumor staging, tissue subtype, and so forth) that may have a significant impact on ICT expression. As a result, the consensus degree between two reviews dealing with the same topic may be modest and reproducibility is not always assured (see Figure 3). Most importantly for the purpose of this review, they do not offer a truly *omic* description since the overall effect of a transportome dysregulation cannot be generally reduced to the sum of the effects reported for individual ICTs.

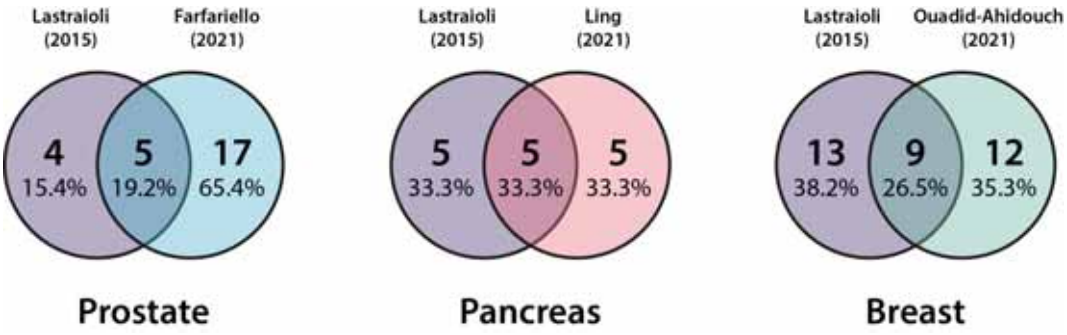

Figure 3. Between-review consensus degree on transportome alterations. *The lists of differentially expressed ion channels specific to three different cancer types from a review by Lastraioli* et al. (21) *were compared with three other lists of ion channel genes from analogous studies published by different authors in 2021 and reviewing transportome dysregulation in prostate cancer* (46)*, pancreatic cancer* (47)*, and breast cancer* (48)*, respectively. In all these three examples, the overlap between the two sets being compared is statistically significant (respective* p*-values = $6.6 \cdot 10^{-5}$, $1.4 \cdot 10^{-6}$, $4.9 \cdot 10^{-7}$, hypergeometric test, with N=350 for the background size of the whole channelome), an indication of the fact that the two studies are addressing similar pathological conditions. However, because of the heterogeneity of the materials and methods used in the underlying primary studies, the number of genes reported as dysregulated by both reviews under comparison is always just a small percentage (20 to 30%) of the global evidence.*

In contrast, this survey will consider just HT transportomics studies, individually providing a comprehensive perspective on the role of ICTs in a particular setting. At the same time, we acknowledge that pooling reviews could be extremely useful as a first and quick reference in everyday research activity. For this reason, we created ICT::bib, a bibliographic database in the form of a browsable GitHub public repository (github.com/TCP-Lab/ICT.bib) where to collect all the papers—including both research articles and reviews—dealing with transportome alteration in cancer and non-cancer models, arranged by research macro-areas.

Why is the transportome being studied?

In this section we provide a brief description of all the transportome-oriented primary studies and meta-analyses we found in literature, arranged by research macro-areas. For each research paper, we report the number of ICTs screened, the wet-lab technology

used to measure their expression levels, the main findings, and the reasons why the omics approach was critical.

Cardiovascular system

In 2005, Gaborit *et al.* (49) isolated RNA from human right atrial samples of patients suffering from valvular heart disease, with or without atrial fibrillation, and used microarrays to explore the transportome remodeling associated with these two pathological conditions. The authors considered 315 genes encoding ICTs and calcium-related proteins using custom-made two-color microarrays. Differential expression analysis (DEA) and subsequent hierarchical clustering revealed a gene signature involving the phospholamban micropeptide and 11 ion channel subunits mediating potassium, sodium, calcium, and chloride currents, specifically dysregulated in patients with atrial fibrillation. Complementing their work, the authors also recorded whole-cell potassium and calcium currents by voltage-clamp experiments to provide functional correlation to expression data. Also, they were able to show that restoration of sinus rhythm in patients with atrial fibrillation was sufficient to reverse the dysregulation of most of the altered ion channels.

In the same years, Harrell and coworkers (50) performed a large-scale RT-qPCR assay to measure the expression levels of 144 ion channel transcripts in the mouse heart during perinatal development compared to the adult stage. The transcriptional differences they observed in each ion channel family largely supported previously known electrophysiological properties of the immature heart (compared to the mature organ) in terms of resting potential, excitability, and action potential properties.

Nervous system

In their recent effort to provide an improved transcriptome assembly of the pond snail *Lymnaea stagnalis'* central nervous system (CNS), Dong and colleagues (51) employed RNA-Seq data about ion channels for a comparative study with other vertebrate and invertebrate model organisms significant to neurosciences. Because of the functional and phylogenetic relevance of the transportome in the study of the CNS, an evolutionary analysis was conducted based on the degree of protein sequence similarity of 211 transcripts putatively coding for ion channels. *L. stagnalis* sequences were compared to their homologs in *Mus musculus*, *Xenopus tropicalis*, *Danio rerio*, *Drosophila melanogaster*, and *Caenorhabditis elegans*. Interestingly, calcium channel family displayed the greatest degree of sequence similarity across all species, suggesting a relative conservation of the related calcium signaling pathways.

An example of study on the peripheral nervous system was provided by Manteniotis *et al.* in 2013 (52) where mRNA was harvested from trigeminal ganglia (TGs) and dorsal root ganglia (DRGs) of adult mice for the assessment of the entire transcriptome by sequencing. Being interested in sensory processes—and pain sensation in particular—the authors focused on both G-protein-coupled receptors and ion channels. Looking at the expression levels of 312 ion channels, they provided a comprehensive characterization of the channelome (i.e., the subset of the transportome comprising all the ion channels) in both TG and DRG samples. In addition, they detected a number of ion channel transcripts previously unreported in mouse TGs, including Piezo2 and several transient receptor potential (TRP) channels.

Osmotic homeostasis

A transportome-wide meta-analysis using RNA-Seq data was carried out in 2021 by Malik and Kim (45) to investigate osmoregulation in the Chinese mitten crab (*Eriocheir sinensis*) under various salinity conditions. The authors collected into a single meta-analysis gill tissue transcriptomic data from four publicly available whole-genome RNA-Seq datasets, finally obtaining a list of 162 differentially expressed ICTs involved in the transition from freshwater to saltwater environment. This allowed them to extend to the entire transportome a gene expression analysis that was usually limited to few well-established markers, such as V-type and Na^+/K^+-ATPases.

In the same year, Durant and colleagues (53) investigated the yellow fever mosquito (*Aedes aegypti*) to provide a clearer picture of ICT expression in anal papillae, the multifunctional organ that mosquito larvae use to adapt to rapid changes in habitat salinity and maintain osmotic balance. The authors conducted an RNA-Seq analysis of larvae abruptly transferred from freshwater (lower salinity) to brackish water (higher salinity) and found 3,747 DEGs. The many ICTs included in this list enabled the authors to present an updated and more comprehensive model for ion, water, ammonia, and xenobiotic transport in mosquito anal papillae.

Also from 2021 is the paper by Knepper and colleagues (54) that collects and reanalyzes transcriptomics and proteomics data produced by the same group as of 2017 (55–58) to draw a comprehensive and hyperdetailed expression map of 431 SLCs along 14 distinct segments of the murine renal tubule. Importantly, all these data have been made public and easily browsable through the website of the Knepper's Epithelial Systems Biology Laboratory.

Interestingly, in 2015 Alvarado *et al.* led a similar investigation using an (apparently) quite distant model organism: the planarian *Schmidtea mediterranea* (59). To establish a comprehensive structure-function map of protonephridia, *S. mediterranea* genome was systematically searched for SLC sequence homology leading to the identification of 318 genes, whose expression patterns were then analyzed by *in situ* hybridization. This analysis showed for the first time the great complexity of planarian protonephridia, pointing at six molecularly distinct segments, and suggesting an extensive functional homology with vertebrate nephrons. Based on these results, the authors proposed planaria as a novel and accessible invertebrate model for the study of human cystic kidney diseases.

Platelets

The first human platelet channelome dataset was published in 2016 by Wright *et al.* who considered 402 ion channel-related proteins and used RT-qPCR to examine their expression (60). The authors highlighted how, despite being anucleate, platelets express a number of mRNAs with as-yet-unknown functional roles in their physiology and pathology. Only 11.4% of the 84 transcripts they found encoding ion channels or regulatory proteins had previously been functionally characterized in platelets.

Gastrointestinal tract

The transportome of the gastrointestinal tract epithelium is a critical player in both healthy and pathological contexts since absorption and secretion are primary functions in this tissue. To our knowledge, the first work explicitly addressing whole transportome expression dates to 2003 (notably, the same year of the Human Genome Project

completion), when Sadée and colleagues used a custom-designed two-color microarray chip featuring 461 probes for transporters and 151 for ion channels relevant to the absorptive capacity of the human intestine (40). The choice of such a technology was aimed to question the reliability of the Caco-2 cell line as an *in vitro* model to test drug absorption from human intestine, based on the many differences in ICT expression that emerged from the comparison of Caco-2 with primary samples of human healthy tissue.

In a study published in 2016 by Pérez-Torras *et al.* (61), human intestinal mucosa samples were excised from patients suffering from Crohn's Disease and their transcriptional profile was compared with those of healthy controls through Illumina GeneChip microarrays. The authors performed a whole-transportome DEA and then focused on SLCs providing a list of 62 differentially expressed genes (from 30 different SLC families) out of the 395 SLCs examined. Consistent with their location in the enterocyte apical membrane, the dysregulated transporters were primarily related to the purinome (i.e., the purinergic signaling apparatus) and amino acid absorption. Remarkably, most of these alterations could be reverted upon sample incubation with *Lactobacillus casei* and *Escherichia coli* commensal bacteria.

Development and regeneration

Fetal growth restriction (FGR) is a pathological condition defined as the failure of the fetus to reach its full growth potential. In 2017, Liu and colleagues (62) investigated the impact of FGR on the proteome of neonatal rat kidneys by means of iTRAQ (isobaric tags for relative and absolute quantification) and 2D LC-MS/MS (two-dimensional nano-liquid chromatography–tandem mass spectrometry). Three years later, the same group revisited this dataset focusing on 22 proteins associated with ICTs out of the 367

differentially expressed proteins detected in the first study (63). Specifically, they discovered that Na^+/H^+ exchanger 1 (NHE1/SLC9A1) was up-regulated in FGR fetuses at both the mRNA and protein levels. Additional *in vitro* functional assays yielded further mechanistic insights, suggesting the involvement of specific endoplasmic reticulum stress regulators, possibly resulting in fetal kidney impairment via a pro-apoptotic pathway.

Another good example of transportome investigation in a developmental model was provided by George and coworkers in (64) where the wing of *Drosophila melanogaster* served as an excellent model to address the key role of ion channels in tissue morphogenesis. In this work, the authors considered 180 different ion channels and examined the wing adult phenotypes using loss-of-function mutants and RNAi lines. This approach highlighted 44—mostly unreported—genes related to ion channels influencing wing development, 31 of them associated with human orthologs often implicated in human channelopathies.

Closely related to development, regeneration studies have also benefited from an omics approach to the transportome, as shown by the work of Levin's group on the role of ion channels and gap junctions in several regenerative models. In (65), for example, Levin and colleagues studied the role of ion fluxes for regenerative patterning in axolotl limbs. First, they collected data about ion channel expression mining a previous transcriptomics dataset by Stewart *et al.* (66), then they used retroviral infections to overexpress a small panel of selected channels in order to manipulate the native membrane potential of axolotl limb blastema cells *in vivo*. Alteration of ion channel expression and/or activity finally resulted in structural defects of the regenerated digits,

thus confirming the hypothesis that electrogenic protein activity is crucial for regenerative patterning.

Stem cells

Mesenchymal stem cells (MSCs) from bone marrow have been studied in different species thanks to their multilineage potential and ability to incorporate into a variety of tissues. Current stem cell research focuses on protein and transcript expression patterns, where ICTs represent interesting candidates, being responsible for the generation of bioelectrical signals, which in turn control the proliferation, migration, and differentiation of various cell types (67). Highly heterogeneous ion channels have been found in different stem cell types, and it is still unclear whether such a heterogeneity is related to different cell subpopulations and/or different phases of the cell cycle. Valuable insights into this scenario have been provided by the works of Li *et al.* on rat (68) and human (69) undifferentiated MSCs from bone marrow, in which the authors used RT-qPCR for a broad-spectrum ion channel screening.

As an alternative to MSCs, Matta and colleagues (70), investigated chondrogenic progenitor cells (CPCs) for regenerative therapies targeting articular cartilage in degenerative diseases. CPCs, indeed, exhibit a potentially superior chondrogenic potential compared to MSCs. Thus, the authors performed a global transcriptomic analysis to study ICTs that may be relevant to chondroprogenitor cell physiology, potentially involved in maintaining the progenitor phenotype under inflammatory conditions. They then focused on the large-conductance Ca^{2+}-activated potassium (BK) channels, being among the most upregulated in CPCs compared to MSCs. Following validation of the microarray data through RT-qPCR, the authors used patch clamp and

dielectrophoresis to characterize the electrophysiological profile of CPCs and provide the first experimental evidence that BK channels are functionally active in undifferentiated CPCs and participate in the maintenance of the chondroprogenitor phenotype.

Cancer

Transportome dysregulation is a common trait of cancer, being associated with the aberrant behavior of cancer cells and providing them with a functional interface to adapt to TME stressors. For this reason, ICTs have been regarded as both promising drug targets and biomarkers for cancer since the early 2000s. In 2004, just one year after their first pioneering ICT omics study (40), the research team led by Wolfgang Sadée hybridized their custom-designed “transportomics microarray” with 60 different human cancer cell lines (the so-called NCI-60 panel) (71) to systematically look for novel drug-transporter interactions that could help in predicting anticancer drug response. Considering the growth inhibitory potencies of 119 common anticancer drugs, they calculated the Pearson correlation coefficient for each gene-drug pair. With this approach, many new potential correlations between ICT expression and sensitivity/resistance to cytotoxic drugs were identified, mainly linking SLCs and ABC transporters to chemosensitivity and chemoresistance, respectively. More recently, other transportome-oriented studies put great emphasis on these two classes of transporters that mediate the uptake and efflux of anticancer drugs with the goal of enhancing tumor responsiveness to chemotherapy and lowering multidrug resistance (35, 36, 72, 73).

In a series of three articles published between 2013 and 2015, Ko and colleagues evaluated the potential of using the expression pattern of ion channel genes as a

genomic biomarker to forecast the prognosis of several human carcinomas, including breast (74), lung (75), and glioma (76). In each case, several Affymetrix microarray datasets were downloaded from public repositories and reanalyzed. Spearman's rank correlation was computed for 280 ion channels and those whose expression significantly correlated with tumor grade were used to define a new cancer-specific molecular signature along with a risk score. Finally, the prognostic power of such ion channel signatures was successfully confirmed in multiple validation cohorts by log-rank test on Kaplan-Meier survival curves and Cox proportional hazard regression.

Two years later, in 2017, starting from whole-genome RNA-Seq data and a list of 425 ICTs (273 ion channels and 152 ion transporters), Pollak and coworkers (77) discovered a set of 25 ion channels significantly overexpressed in glioblastoma stem-like cells from human glioblastoma multiforme (grade IV glioma) samples compared to normal neural cells. They found this set of genes to be linked to the prognosis of glioblastoma and highly impacting on glioblastoma stem-like cells viability, thus suggesting for these channels a large therapeutic potential against tumor growth. Importantly, the authors chose to focus just on those ion channels that were selectively expressed in glioblastoma stem-like cells and not in other cell types, with the aim of minimizing toxic off-target effects in the therapeutic clinical setting. The omics approach, in this case, allowed the authors to reduce functional redundancy by clustering dysregulated ICTs in a few classes of functionally related channels (e.g., epithelial Na^+ channels, $GABA_A$ receptors, Ca^{2+}-activated K^+ channels, inwardly rectifying K^+ channels, and so on). Interestingly, the overlap between the latter two similar studies (76, 77) in terms of dysregulated ICTs was quite low, confirming that subtle differences in boundary conditions (i.e., the use of stem-like cells from glioblastoma multiforme *vs*

bulk tissues, different workflows and analysis pipelines, …) can have a significant impact on the observed transportome configuration and hinder reproducibility.

As of 2016, Schwab and coworkers have pursued the study of ICT alterations in many different experimental models related to pancreatic ductal adenocarcinoma (PDAC), including *ex vivo* samples of microdissected tumor epithelium (78), pancreatic stellate cells (79), and human PDAC A818–6 cell line (80). In (78) in particular, starting from a whole-genome expression profiling by microarrays, DEA was performed on a subset of 840 genes representing the entire transportome. As a result, the authors identified an ICT gene signature related to PDAC development that could explain the impairment of some of the most distinctive functions of the pancreatic duct epithelium during tumor pathogenesis. Indeed, KEGG analysis mainly correlated the ICT genes found to be dysregulated in PDAC with specific biological processes like alkaline, acetylcholine, and insulin secretion, tissue homeostasis, water transport, and detoxification of ROS as well as the regulation of cell polarity. Interestingly, in (80) a transportome-scale expression screening was carried out performing an *nCounter* assay, a recent technology developed by nanoString that enables the direct count of gene product copies (i.e., without any PCR amplification step) for a panel of selected targets, such as all the ICT transcripts.

Yeasts and Fungi

The research on recombinant microorganisms' transportome may boost the industrial productivity of small molecules for pharmaceutical, food, and materials applications by exploiting the mechanisms of their natural secretion from microbes, instead of their more expensive separation and purification. For instance, in 2017 Mans and colleagues

(81) measured lactate production rates from a CRISPR/Cas9-edited *Saccharomyces cerevisiae* strain featuring 25 deletions in genes coding for transporters, with the aim of identifying those essential for lactate production. In 2021, Wang *et al.* (82) applied to *S. cerevisiae* a similar top-down strategy for transporter disruption to develop a high-throughput workflow for the identification of transporters involved in the production of an arbitrary target metabolite. Starting from a panel of 411 transporter genes, the authors identified those involved in *cis,cis*-muconic acid, protocatechuic acid, and betaxanthins secretion. A recent computational investigation by Claus *et al.* (83) led to the whole-transportome profiling of the biosurfactant-producing yeast *Starmerella bombicola*, identifying 254 putative ICTs (5.49% of its genome). In addition, by means of RNA-Seq, they proposed the ABC transporter superfamily as a good candidate to explain the high efficiency of *S. bombicola* for sophorolipids (SLs) export, also discovering a second SL exporter (SbSLMdr.2) other than the already well-established SbSLMdr.1. Based on these premises, four deletion mutants were created and functionally characterized to identify ABC transporters involved in the efflux of medium chain fatty alcohols.

Noteworthy, besides the field of synthetic biology, the study of the transportome in yeast has also attracted the attention of biomedical researchers, as evidenced by the bioinformatics studies by Prasad and colleagues on the ABC superfamily (84) and the major facilitator superfamily (MFS) (85) in *Candida glabrata* and *Candida albicans*, respectively.

Many different fungal species other than yeasts have been subject to transportomics approach by different research groups. Some examples include *Aspergillus niger* and *Trichoderma reesei*, for their industrially relevant ability to secrete

lignocellulose-degrading hydrolytic enzymes (86, 87); *Laccaria bicolor*, to elucidate the mechanisms that make it such an efficient nitrogen supplier to host plant in symbiotic interactions (88); *Pucciniales*, since they represent harmful plant pathogens responsible for rust diseases and crop damage (89).

Bacteria and Archaea

Escherichia coli is a genetically diverse species causing urinary tract infections, gastroenteritis, pyelonephritis, and hemorrhagic colitis in hundreds of million people worldwide annually. In 2014, the team led by Milton Saier Jr.—the founder and principal curator of the Transporter Classification DataBase (TCDB) (90–94)—examined seven well-characterized *E. coli* pathogens and compared them with each other and with the non-pathogenic *E. coli* K12 strain to identify ICT proteins specifically related to pathogenesis (95). More in detail, Saier and colleagues performed a computational analysis of sequence similarity looking for the occurrence of transporter-associated or channel-forming protein domains. These studies revealed that each pathogenic strain expresses a characteristic set of protein secretion systems used to inject effector molecules into the host cell. In the following years, Zafar and Saier conducted many others comparative genomic studies addressing transporter proteins in probiotic and pathogenic strains of *E. coli* and *Salmonella enterica* (2017) (96), *Bacteroides* (2018) (97), *Treponema* (2019) (98), *Lactobacillus* (2020) (99), and *Bifidobacterium* (2021) (100). In parallel, the same research group carried out many ICT-related evolutionary studies, among which the full transportome characterization of the Asgard archaeal superphylum—maybe the closest archaeal ancestor to Eukarya—specifically

investigating its evolutionary profile and highlighting the importance of the proton electrochemical gradient for secondary solute carriers and ATP synthesis (101).

Taken together, these studies provided valuable knowledge about the transportome contribution to bacterial pathogenic potential, possibly paving the way to the design of novel anti-microbial drugs for the prevention and control of antibiotic resistance spread, one of the biggest public health challenges in the coming years.

Plants

The transportome—in particular ABC transporter superfamily—plays a crucial role in plant metabolism, development, homeostasis, and osmotic regulation by controlling nutrient intake as well as water and cation fluxes (102, 103). Moreover, it is of relevance for environmental pollution, since toxic metals accumulated in the soil can be effectively absorbed by plants, as shown by Gill and coworkers with their study on *Brassica napus* under chromium stress conditions (104).

In 2011, Monneuse and colleagues (105) set up a targeted proteomics approach for the large-scale profiling of plant membrane transporters. Applied to the transportome of *Arabidopsis thaliana*, this method allowed the simultaneous quantification of 9 plasma membrane intrinsic proteins (PIPs) from leaves and 13 PIPs, 3 H^+-ATPases, 2 ammonium transporters, and 2 nitrate transporters from roots, under both normal and salt stress conditions. Working on the same biological model, in 2018 Zhang and coworkers (106) leveraged a multi-targeted transportome screening to identify the ICTs having a role in plant growth and development. They used a transporter-specific library of 1,777 artificial-microRNAs (amiRNAs) that allowed them to systematically target all the (currently known) families of transporters in *A. thaliana*, overcoming the functional

redundancy issue affecting classic forward genetic studies in plants. Measuring the silencing effects of the different amiRNAs on shoot growth phenotypes, the researchers identified two previously unreported ABC transporter genes (ABCB6 and ABCB20) that subsequent functional assays showed to play an important role in basipetal polar auxin transport. Notably, the further implementation of this strategy has recently led to the development of Multi-Knock, the first multi-targeted CRISPR toolbox allowing for a transportome-scale functional analysis of *A. thaliana* (107).

In 2021, Pinto and colleagues (108) analyzed the transportome of *Coffea canephora*, a perennial plant belonging to the *Rubiaceae* family and genus *Coffea*, which contains more than 120 species, including *Coffea arabica*, whose seeds are ground and infused to produce coffee, one of the most popular beverages all over the world. Among the 25,574 protein-coding genes annotated in the genome, the authors identified 1,847 putative membrane transporters, some of them potentially involved in the accumulation of specialized metabolites associated with beverage quality and bioactivity attributes.

Symbiotic systems

In recent years, the transportome has gained growing interest as a promising key to better elucidate the complex interface dynamics among symbionts in mycorrhizae, lichens, and rhizospheres. ICTs play indeed a pivotal role in facilitating nutrient uptake and exchange across the membranes in symbiotic systems. For instance, in arbuscular mycorrhizal (AM) symbiosis, transporters are crucial for the uptake and exchange of nutrients, among which phosphorus and nitrogen, two essential elements in plants. In 2017, Calabrese and colleagues (109) analyzed the *Populus trichocarpa–Rhizophagus irregularis* symbiosis transcriptome focusing on nitrogen starvation effects. They

sequenced the mRNA from both mycorrhizal and non-mycorrhizal *P. trichocarpa* (poplar) roots and found that nitrogen limitation led to a general induction of fungal transporters (ammonium, phosphate, nitrate, amino acid, and ABC superfamily transporters) suggesting an increased nutrient demand by the host plant. The broad host range of AM fungi also prompted their following study in 2019 (110), which focused on the transportome in a mycorrhizal network. The transportome of *R. irregularis* associated to the poplar *P. trichocarpa* and *Sorghum bicolor* (sorghum) was investigated under high and low phosphate availability, using RT-qPCR, RNA-Seq, and metabolome analysis. In these experiments, fungus was shown to differentially modulate its metabolism when interacting with the two different plant species, inducing the expression of specific phosphate and ammonium transporters in plants.

In the same year, Armaleo and colleagues (111) reported the first parallel genomic analysis of both organisms in a lichen made up of the fungus (mycobiont) *Cladonia grayi* and the alga (photobiont) *Asterochloris glomerata*. RNA-Seq reads from the aposymbiotic fungus, the aposymbiotic alga, and a coculture of the two were analyzed focusing on predicted genes or proteins of potential symbiotic significance. Moreover, starting from the analysis of *C. grayi* transportome, they searched for lineage-specific expansions or contractions of multigene families and identified two fungal transporters potentially central in the carbon and nitrogen exchange at the symbiotic interface, namely an importer for ribitol (the carbon source provided by trebouxoid algae to their fungal partners) and a possible ammonium exporter that pointed at NH_4^+ as a major nitrogen source provided by the mycobiont to the photobiont.

Another relevant symbiocosm system is represented by the rhizosphere, a restricted region of soil that comprises complex interacting communities of fungi,

bacteria, and animals, which establish a symbiotic relationship with the plant biomass above and below the soil. For example, it has been shown that *Sinorhizobium meliloti* bacteria can express different ABC and TRAP transporters for the uptake of many growth-limiting substances from the rhizosphere, thus promoting plant growth (112). In 2014, Larsen and coworkers (113) defined a novel metric called Predicted Relative Transmembrane Transport (PRTT) to quantify the relative ability of an organism to transport specific metabolites across the cellular membrane. PRTT was computed from the genome of different Pseudomonas strains and then used to train Support Vector Machine (SVM) models, ultimately proving to be the best parameter to predict both human pathogenicity (113) and Pseudomonas ecological role (or niche) in the rhizosphere (114). Complementing these results, a 2017 follow-up study from the same group (115) deepened the relationships between the predicted transportomic capacity of four different Pseudomonas strains—calculated again as PRTT scores—and their plant growth-promoting effects on *Populus tremuloides* (trembling aspen).

Theoretical

The omics approach to the transportome also proved to be well suited for pure computational and theoretical studies such as that carried out by Darbani *et al.* (116) where the transportome composition of 249 species across the different domains of life (bacteria, archaea, primitive eukaryotes, plants, fungi, and animals) was systematically analyzed to draw conclusions about energy requirements in an evolutionary perspective. This paper showed the progressive shift from ATP-dependent to low-energy-demanding transporter families (i.e., carriers and ion channels) when moving from prokaryotes to eukaryotes. Notably, such a negative selection of the more energetically expensive

transporter systems—ABCs in particular—could be quantified through the *energy usage efficiency* (EUE) of the transportome, defined as the average ATP-usage per single transport cycle (i.e., per single substrate translocation). EUE was evaluated for all the 249 representative species showing an energy consumption improvement in animals compared to bacteria of about 0.5 ATP molecules per single transport event. In the same report, based on the analysis of the mitochondria-specific solute carriers (i.e., the SLC25 family), Darbani and colleagues provided new evidence for the *Rickettsiales* order as the most likely origin of the eukaryotic mitochondria.

Among the many studies presented in this section, some have proven to be particularly relevant from a physiological point of view, as they were able to push scientific inquiry beyond the mere descriptive level and delve into mechanisms through the investigation of the transportome. A selection of eight is depicted in Figure 4, whereby for each selected paper a sentence summarizes the functional aspect elucidated in it, with many direct links to the different subclasses of ICTs involved.

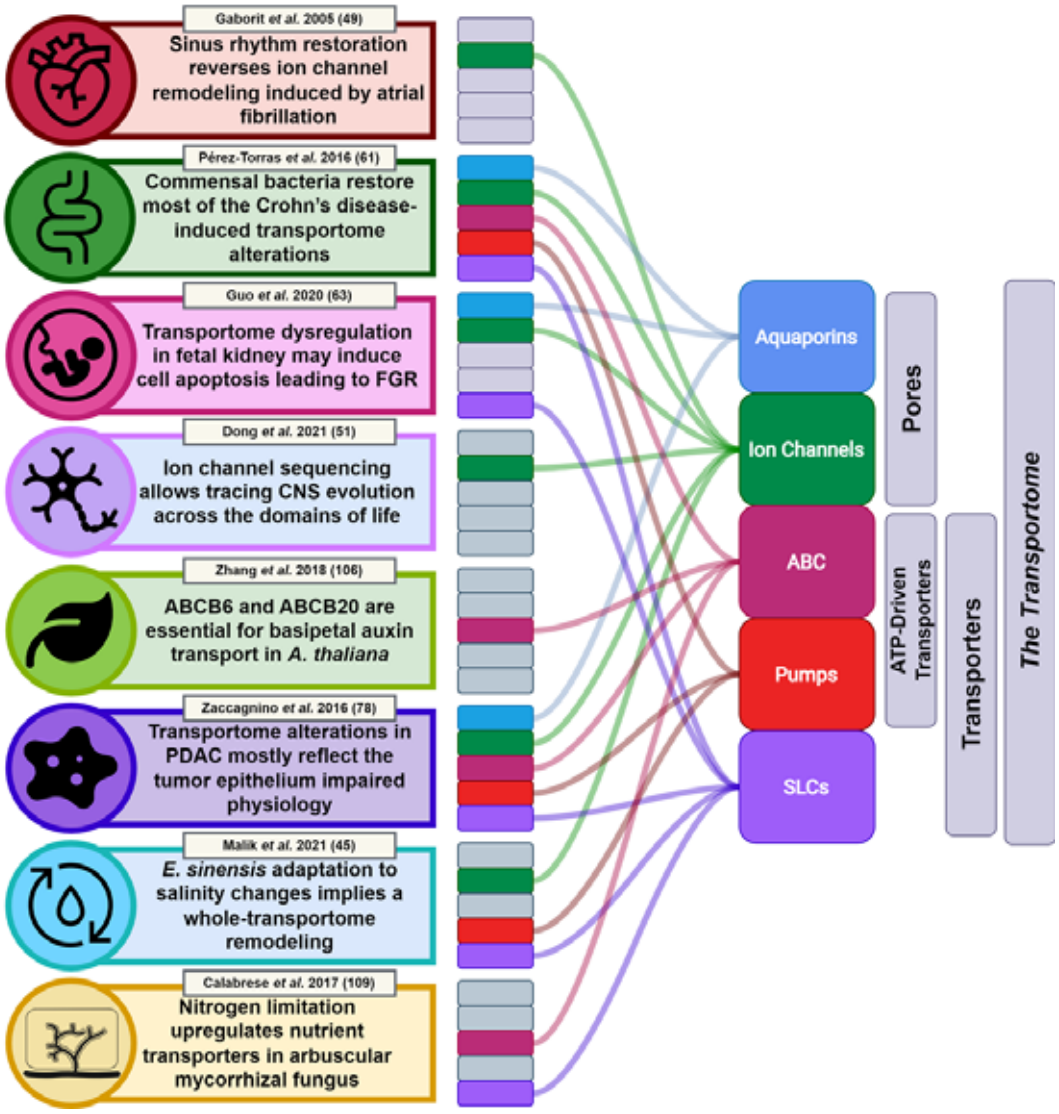

Figure 4. Transportomics as a novel effective paradigm for physiology. *Eight exemplificative studies particularly relevant for the physiological, functional, or mechanistic insights they provide are listed on the left and connected with the corresponding subclasses of ICTs shown to be involved in those experimental conditions. Bibliographic reference numbers are given in round brackets. FGR: Fetal Growth Restriction; CNS: Central Nervous System; PDAC: Pancreatic ductal Adenocarcinoma.*

Where to find all the ICTs you need?

Getting an updated and accurate list of all the ICTs expressed in your experimental model is the first crucial step for the study of the transportome. This is true whether you decide to go through a whole-genome screening (by microarray or RNA-Seq) and then

focus on the transportome, or you choose to directly address a panel of genes of interest (by RT-qPCR or nanoString technology). Fortunately, the Web offers us a wide range of advanced tools that can be applied for this purpose. This section is intended to give the reader useful references and some operational cues for transportome screening.

The HUGO Gene Nomenclature Committee (HGNC), the official source for human gene symbols and names, is an important resource in omics investigations for those who work with human samples (www.genenames.org, (117, 118)). The HGNC database can be searched by single entry to retrieve individual gene symbol reports, or through the *multi-symbol checker* tool to retrieve the most up-to-date gene symbols for any given gene list and so avoid possible issues caused by aliases or obsolete gene names when comparing (i.e., intersecting) different gene sets. Most importantly, the HGNC database can be interrogated even by *gene groups*, among which are defined those of *ion channels* (including aquaporins), *solute carriers (SLCs)*, *ATPases*, and *ATP binding cassette transporters (ABCs)*. Importantly, if your interest is limited to human solute carriers, another indispensable Web reference is the database of *SLC Tables* (slc.bioparadigms.org, (119, 120)), curated and maintained by Matthias A. Hediger, current special advisor on SLCs to the HGNC. This site provides accurate and up-to-date information on all human solute carriers so far identified, according to their official system organization into 66 gene families within the SLC superfamily.

Another essential online resource providing a comprehensive catalogue of human ICTs is the database of the International Union of Basic and Clinical Pharmacology (IUPHAR-DB) (121, 122) available at www.guidetopharmacology.org. From the home page, selecting the section heading *Targets*, the user can access a hierarchical

representation of all ion channel and transporter families. For any ICT of interest, detailed information about agonist and antagonist ligand molecules and their specificity are provided. The database is based on the popular publication *Concise Guide to PHARMACOLOGY (CGTP)* published in the British Journal of Pharmacology (123), formerly popular among physiologists as the *BPS Guide to Receptors and Channels (GRAC)* (124).

Any non-human but still widely adopted model organism—such as mouse, rat, chicken, *Xenopus*, zebrafish, *Drosophila*, *C. elegans*, *Arabidopsis*, *S. cerevisiae*, or *S. pombe*—has a corresponding specialized nomenclature committee, which provides online resources similarly to HGNC (e.g., www.informatics.jax.org, (125) for mouse). Interestingly, if you are dealing with species other than those mentioned above, it could be helpful to explore the Vertebrate Gene Nomenclature Committee (VGNC) database, an extension of the HGNC project to a selection of vertebrate species that do not have a dedicated gene nomenclature committee, including chimpanzee, macaque, horse, cattle, pig, dog, cat, and several other species (vertebrate.genenames.org), (117, 126). In addition, both HGNC and IUPHAR databases provide tools and features for homology/orthology detection or prediction across multiple species.

The Transporter Classification (TC) system supported by the International Union of Biochemistry and Molecular Biology (IUBMB) is another powerful resource for transportome investigation (90–94). Notably, the TC system classifies all known ICTs from all living organisms, based on protein structure, functional features, and phylogenetic information. The related database (TCDB)—created, maintained, and curated by the Milton Saier Lab—is freely accessible at tcdb.org and it currently (July 2023) consists of more than 23,100 protein sequences, organized in about 1850

transporter families, where family membership implies similar function and mechanisms, regardless of the target species. At the top of TC system hierarchy are seven major classes: TC1 for *Channels/Pores*, TC2 for *Electrochemical Potential-driven Transporters*, TC3 for *Primary Active Transporters*, TC4 for *Group Translocators*, TC5 for *Transmembrane Electron Carriers*, TC8 for *Accessory Factors Involved in Transport*, and TC9 as a residual class for *Incompletely Characterized Transport Systems.* Classes TC6 and TC7 are currently empty and reserved for possible additional classes of transporters yet to be discovered. The TCDB is an extremely powerful biochemistry-oriented classification tool, but it might appear overly complex for most of the cell physiologists who only wish to explore transportome expression in one conventional experimental model. As an alternative to direct TCDB access, TransportDB 2.0 (www.membranetransport.org/transportDB2) represents a more user-friendly option to browse the transportome across all the domains of life according to the TC system (127).

Much more popular among bioinformaticians, the Gene Ontology (GO) knowledgebase by the GO Consortium (geneontology.org, (128, 129)) has evolved into a crucial tool for the interpretation of omics data and may also prove useful when it comes to the transportome. GO is a formal structure based on directed acyclic hierarchical graphs (DAGs) aiming at the multiscale description of biological systems. Just like the TCDB, GO is species-agnostic, but it does not focus on ICTs only. Instead, GO provides three independent representations of the current knowledge about *Cellular Components* (CCs), *Molecular Functions* (MFs), and *Biological Processes* (BPs), in which each GO term is annotated with one or multiple genes reported or predicted to play a role in it. To get a comprehensive list of ICTs, the user can resort to AmiGO 2—the user-friendly Web tool for browsing ontologies and related gene product annotations

(amigo.geneontology.org/amigo, (130))—and search for the MF GO term *GO:0015075* (*monoatomic ion transmembrane transporter activity*), or for its parent—and more general—term *GO:0022857* (*transmembrane transporter activity*) (Gene Ontology release 2023-06-11). A filter can be applied to narrow the results to a particular organism and/or to select a specific family of ICTs.

Those who are uneasy with GO representations can alternatively resort to PANTHER (Protein Annotation THrough Evolutionary Relationship, (131–133)) at www.pantherdb.org. PANTHER is a classification system for genes and gene products that focuses on the concepts of gene families and subfamilies, defined on evolutionary and functional bases. Being part of the Gene Ontology Reference Genome Project (134), PANTHER is closely related to GO and uses a subset of GO terms to implement a simplified ontology (called *PANTHER GO-slim*) and an ontology browser (known as the PANTHER Prowler, accessible from the home page via the *Browse* tab) that enables users to quickly find genes searching by *protein classes*, usually named according to the biological function they represent. A complete list of ICT genes can be obtained selecting the first level protein class *transporter (PC00227)*—which includes in turn the subcategories *ion channel*, *primary active transporter*, and *secondary carrier transporter*. In addition, the *Gene List Analysis* tab from PANTHER home page is a great tool for inspecting the relative proportion of dysregulated ICTs within a DEG list and thus evaluate the relevance of the transportome in a particular experiment; just paste your gene list and select *Functional classification viewed in graphic charts*, then choose *Protein Class* from the drop-down menu and look for the *transporter* bar. PANTHER is also a member of the InterPro consortium (www.ebi.ac.uk/interpro, (135)) and a major contributor to the InterPro integrated database for functional classification of protein

families (other important member databases being Pfam, PROSITE, SMART, CDD, and TIGRFAMs).

The gold standard reference for protein sequence for a variety of organisms, including bacteria and viruses, is UniProtKB (Universal Protein KnowledgeBase, www.uniprot.org). This resource can be used when the transportome needs to be studied at the protein level, potentially accounting for isoforms resulting from alternative splicing or post-translational modifications (136). Each entry is associated with either a manually curated (Swiss-Prot) or a computationally generated (TrEMBL) functional annotation. As well as most of the other databases reviewed in this section, UniProtKB also allows retrieval of families of functionally related genes (through the feature *Keywords* implemented in the *Supporting data* section).

It is also important to keep in mind that most of these databases cross-reference each other. For instance, PANTHER, InterPro, and UniProtKB contribute to the GO database, while UniProtKB uses InterPro to automatically classify and annotate the domains of the TrEMBL entries. Notably, cross-links between databases are at the heart of GeneCards (www.genecards.org, (137)), a gene-centric human data aggregator that automatically retrieves information from 185 sources (July 2023), including HGNC, IUPHAR-DB, IUBMB, GO, STRING, NCBI Entrez Gene, Ensembl, InterPro, UniProtKB/TrEMBL, PubMed, GTEx, and many others. GeneCards thus offers a unified point of entry for a variety of other databases, integrating genomic, transcriptomic, and proteomic data with genetic, clinical, and functional information. GeneCards' search box can be used to obtain a single gene report—a sort of an identity card where all the information collected is rearranged in 18 distinct sections—but it also enables users to formulate more complex queries by specifying the gene family of interest and/or

narrowing the search to some sections. In particular, it is feasible to obtain a thorough list of human ICTs by searching for *[functions] (transporter OR (ion AND channel))*. However, since there is no assurance that the bare existence of those terms inside the *Function* section equates to an actual ICT entry, the aforementioned lists frequently have a tendency to be overinclusive and necessitate manual filtering.

Finally, to cope with the fragmentation of these Web resources, offer a comprehensive repository of biophysically and physiologically reliable ICT annotations, and provide an integrated environment for transportomics research, our team has recently started an Open Science project (138), which consists of a SQLite database–called the Membrane Transport Protein DataBase (MTP-DB)–collecting information about human ICTs from the most important consortia reviewed in this section (github.com/TCP-Lab/MTP-DB), and a software suite for the automatic generation of meaningful ICT lists and the evaluation of their possible enrichment in gene expression datasets (github.com/TCP-Lab/transportome_profiler).

Limitations and perspectives

With a few rare exceptions, all the transportomics studies we discovered in the literature assessed ICT gene expression by measuring mRNA levels by microarrays, RNA-Seq, RT-qPCR, or directly via nCounter technology, almost completely ignoring their related protein products. This is mainly due to the field of proteomics being considerably younger than transcriptomics, from both a theoretical and technological point of view. On the theoretical side, it is worth noting that the first draft of the human proteome dates to 2014 (139), while the Human Proteome Project (HPP) (140) coordinated by the

Human Proteome Organization (HUPO)—though at a very advanced stage—is still an ongoing project (141). As for the experimental side, the systematic HT profiling of cell protein expression is a particularly tough challenge when it comes to the transportome because of the low average abundance of many ICTs, their hydrophobic features, and their instability when pulled out of the lipid bilayers (120, 142, 143). These factors make it necessary for ICTs—and membrane proteins more generally—to be detected and accurately quantified using highly sensitive mass spectrometry (MS) techniques and special preprocessing procedures (144–146).

These technological limitations in membrane protein detection and quantification are particularly relevant since the relationship between the transcriptional and translational levels is typically not trivial, both in physiological (see e.g., (147)) and pathological contexts, like that of cancer, where ribosome biogenesis is subjected to complex transcriptional alterations (148–151). Based on such scientific evidence, transcriptomics and proteomics should be regarded as two complementary rather than alternative tools in systems biology. Accordingly, a more thorough integration of these two omics is desirable and is expected to soon provide new insights into the complex regulatory chain that links genes to the molecular functions they represent (see for instance the TIMEOR proposal (152) as a possible way to bridge this gap).

Other restrictions connected to the current proteomics standards include the ability to distinguish between different proteoforms and provide information on their cellular localization (153). Indeed, it is crucial to keep in mind that, even though most proteins—including ICTs—can exist in a variety of proteoforms due to posttranslational modifications that are essential for their proper operation, standard MS methods for HT proteomics rely on short proteolytic peptides (typically less than 20 amino acid residues)

that make it difficult to distinguish between these different forms. This largely explains why the first deep proteome sequencing enabling the global detection of human proteoforms was only published in March 2023 (143). Alternative approaches for intact protein HT sequencing, including the so-called “top-down” and “middle-down” strategies, are, however, currently being developed (154, 155).

Beyond translation, even the targeting of the newly synthesized membrane proteins needs to be carefully considered, being a functional prerequisite of any ICT, at least to properly exert what is considered their primary function. Notably, it has been suggested that ion channel mislocalization plays a role in a number of clinical diseases (see e.g., (156)). To address this issue, preliminary steps for integral membrane proteins enrichment have been proposed (146).

Finally, even when properly translated and targeted, ion channels and electrogenic transporters (i.e., about 25% of SLCs, (157)) need to be functionally characterized by means of fluorescent indicators or electrophysiology, with the gold standard still being the traditional patch clamp, an extremely powerful but low throughput technique (for electroneutral SLCs the picture is even worse; a nice overview of the measurement techniques currently available to determine SLC activity can be found in (157)). Nevertheless, following the latest technological improvements (i.e., the so called “robotic” or automated patch clamp (158, 159)), we are likely to be on the edge of a revolution, which might make ion channels even more attractive targets of research (24).

Taken together, these considerations highlight the often-overlooked distance between a protein-coding DNA sequence and the corresponding functional protein, and warrant caution on inferring functional meanings from transcriptional alterations,

especially when dealing with the transportome. On the other hand, we can think of all these restrictions as something contingent, driven by the current state of technology, and, as such, supposedly transient. In any case, transportomics may appear to be still largely descriptive at the moment, mostly profiling gene expression without claiming to penetrate the underlying mechanisms. This is not completely true though, as the majority of the studies we discussed here provided crucial background knowledge for later mechanistic insights, usually focused on a reduced number of ICTs. In some cases, functional assays were conducted by the same authors in a subsequent—or even in the same—paper to elucidate the physiological implications of transportome alterations (see, e.g., (49, 61, 63, 83, 106), and Figure 4). In most cases, however, the initial transportomic screening enabled the generation of new well-supported hypotheses about the biophysical and physiological mechanisms which other research teams could then test. For example, the 2007 comparative study by Harrell and coworkers—addressing channelome transcriptional changes in mouse heart development (50)—served as a starting point for a 4-year later paper (160) in which Cai and colleagues performed whole-cell patch clamp experiments to investigate the developmental changes of the voltage-dependent sodium current in human heart. Another example is the paper by Manteniotis *et al.* (52) in which they described the transcriptional specificities of mouse DRGs and TGs in terms of ICTs and GPCRs. Even neglecting GPCRs, this paper was later used as a reference transcriptional framework for many pharmacological studies, mainly aimed at finding new analgesic drugs and focusing on single channel families, such as voltage-gated sodium channels (161), acid-sensing ion channels (162), two-pore domain potassium (K_{2P}) channels (163–165), G protein-

activated inward rectifier potassium ($K_{ir}3$) channels (166), and hyperpolarization-activated cyclic nucleotide-gated (HCN) channels (167).

A final caveat we wish to make about the entire transportomics investigation concerns the degree of reproducibility of the findings, which may be low in the case of not perfectly comparable experimental models, as already suggested by the two studies on glioblastoma multiforme reported in the *Cancer* subsection (76, 77). More generally, because of the critical interface role ICTs play in homeostasis and cell adaptation to changing environments, it is reasonable to expect high sensitivity of these genes to subtle environmental factors (such as temperature, pH, osmolarity, nutrient availability, and others) even when working with a single *in vitro* model, under (apparently) tightly controlled conditions. Unfortunately, a rigorous and systematic quantification of these effects, to our knowledge, has never been done.

Conclusions

Our review of the scientific literature reveals that the interest in the transportome, and particularly the study of its expression and functional dynamics under physiological conditions or human diseases, cuts across many different areas of investigation, involving in turn an extremely wide range of biological models. Despite their apparent distance, all the research groups mentioned in this review share the same interest in the transportome, organically conceived as the cellular machinery responsible for transmembrane transports, and, most importantly, they agree that only an omics approach has the potential to yield a meaningful picture of the biological processes under study, within the irreducible complexity of the cell.

We hope the present work may attract some more traditional physiologists towards the omics paradigm, while fostering discussion among all the researchers already involved in it, since they have been facing the same methodological challenges and could benefit from the same technological advancements and analytical tools.

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